REMARKS

Claims 1, 5-9, 11, 12, 28-33, 37-43 and 45-48 are pending and remain rejected following entry of Applicants' Amendment After Final of November 12, 2009 (see Advisory Action mailed 12/1/09). Claims 2-4, 10, 13-27, 34-36 and 44 were previously cancelled without prejudice or disclaimer.

Claims 6, 11, 29 and 42 are cancelled herewith without prejudice or disclaimer. Claims 1 and 9 have been amended to incorporate the limitations of claims 6 and 11 and 29 and 42, respectively, solely in an effort to expedite the allowance of the application. Support for the amended claims can be found throughout the specification and in the claims as originally filed. Applicants reserve the right to pursue previously claimed subject matter in one or more continuing applications. No new matter has been introduced by the foregoing amendments.

Claims 1, 5, 7-9, 12, 28, 30-33, 37-41, 43 and 45-48 are pending following entry of the instant amendment. With respect to the rejections maintained by the Examiner, Applicants respectfully request reconsideration and examination of this application and the timely allowance of these claims in view of the arguments presented below.

I. Interview Summary

The Applicants' wish to thank Examiner McGarry for the personal interview kindly granted to Applicants' representatives, Debra Milasincic, James Velema and Briana Erickson, on January 20, 2010. During the interview, the outstanding §103 rejection was discussed (see Examiner Interview Summary of 1/27/10). In particular, the arguments of record concerning the unpredictability of single nucleotide discrimination at the time the application was filed were discussed. Although an agreement regarding the allowability of any of the pending claims was not reached, the Examiner agreed to consider additional evidence of nonobviousness which is provided below.

II. Rejection under 35 U.S.C. § 103(a)

In the Advisory action the Examiner has maintained his rejection of claims 1, 3-9, 11, 12, 28-33 and 35-40 under 35 U.S.C. §103(a) as being unpatentable over Tuschl *et al.* (US 2004/2059247 A1), Elbashir *et al.* (The EMBO Journal Vol. 20(23), 2001), Klug *et al.* (European Journal of Physiology, Vol. 441 (6 suppl): R205, 2001), Brown *et al.* (WO

94/19493), Siddique *et al.* (Neurology Vol. 47 (suppl 2): S27-S35, 1996), and Kunst *et al.* (Nature Genetics Vo.. 15:91-94, (1996)). The rejection of claims 41-48 under 35 U.S.C. § 103(a) as being unpatentable over Tuschl *et al.*, Elbashir, Klug *et al.*, Brown *et al.*, Siddique *et al.*, and Kunst *et al.* as applied to claims 1-12 and 28-40 above, and further in view of Brummelkamp *et al.* (Science Express, 21 March 2002) was also maintained.

In particular, the Examiner alleges that "[t]he prior art relied upon by the Examiner has provided a means and motivation to provide for siRNA compounds that would provide allele specific inhibition." (see Advisory Action, Note 11).

Applicants respectfully traverse this rejection and provide the following response to supplement the previous arguments filed on November 12, 2009.

(a) No Reasonable Expectation of Single Nucleotide Allelic Discrimination Using siRNA or other RNAi Agents Existed in the Art

In their previous response, Applicants submitted additional evidence (the Holen, Jacque *et al.*, Yu *et al.*, Hamada and Boutla *et al.* references) which supports the conclusion that one of skill in the art at the time of Applicants' filing date would have lacked a reasonable expectation that single nucleotide allelic discrimination was achievable with an siRNA or other RNAi agent. In his Advisory Action, the Examiner indicated that this additional evidence had not been considered. Moreover, the Examiner indicated that "the claimed invention is not even limited to there being one nucleotide difference in the compounds used" and that the claims are "broad enough to include the inhibition of any differing allele in a cell" (see Item 11 of Advisory Action).

In response, Applicants have amended the claims to incorporate the features of pending claims 11, 16 and 29. The claims now specify that the siRNA is "matched completely with a mRNA encoded by the mutant allele but comprises a nucleotide mismatch with a mRNA encoded by the wild-type allele" and that the siRNA "is capable of single nucleotide discrimination". Applicants respectfully request that the Examiner now consider the proffered evidence and the following arguments in the context of the currently presented claims.

As Applicants noted in their previous response, the Tuschl and Elbashir references describe the same standard gene-specific silencing experiment, wherein sequence changes are introduced into the paired segments of siRNA duplexes to examine the effects of these sequence changes on the efficiency of silencing a single target gene sequence (a firefly luciferase reporter sequence). Applicants maintain their position that one skilled in the art would have had no reasonable expectation that single nucleotide discrimination between a wild type and mutant alleles of the SOD1 gene could be achieved using siRNA technology at the time the instant invention was made. Applicants submit that the single experiment[1] described by Tuschl *et al.* and Elbashir *et al.* is speculative at best and fails to demonstrate that single-nucleotide siRNA discrimination among two related alleles of the same gene was realistically achievable at the time of the invention.

Moreover, one of skill in the art at the time the application was filed would have seriously doubted the applicability of the Tuschl/Elbashir experiment given the publication of several contemporaneous reports suggesting that siRNAs were in fact generally tolerant of sequence changes that introduce single-base mismatches between the siRNA and its target. For example, Holen *et al.* (*Nucleic Acids Research,* 30(8): 1757-66 (2002) synthesized siRNA to target sites within the mRNA of human tissue factor (TF), and observed that the wild-type siRNA *hTF167i-wt* exhibited 80% silencing capability. By comparison, the mutant siRNA *hTF167i*-M1 with a single-nucleotide **"central"** mismatch (see Figure 6A) "exhibited 65% silencing capability", leading the authors to conclude that "RNAi to a certain degree tolerates siRNA: mRNA mismatches" (page 1765). Thus, although the mismatch was chosen to be "maximally disruptive" as in the Tuschl/Elbashir experiment (page 1763, first column, second paragraph, lines 1-10) the authors demonstrated that it only partially reduced the rate and extent of target depletion (page 1765, column 1, paragraph 4, lines 12-15).

Jacque *et al.* (*Nature*, 418: 435-438, 2002), Yu *et al.* (*PNAS*, 99: 6047-6052, 2002) and Hamada *et al.* (*Antisense and Nucleic Acid Drug Development*, 12: 301-309, 2002), provide further evidence that the state of the art was unsettled as of Applicants' filing date. Jacque *et al.* directed siRNA duplexes against several regions of the HIV-1 genome, including the viral long

[1] Applicants note that the Tuschl *et al.* patent publication and Elbashir *et al.* research article disclose the same experimental results.

terminal repeat (*LTR*). *LTR* was targeted with both the wild-type siRNA *TAR* and the single-nucleotide mutant *MTAR*, both of which suppressed reverse transcription activity to nearly the same extent (page 435, Figure 1b). Similarly, Yu *et al.* observed that hairpin RNA (shRNA) possessing a single mismatch relative to the *luc-GFP* target silenced the reporter to nearly the same extent. Any difference was within the margin of error (page 6048, Figure 2c). Finally, Hamada *et al.* found that wild-type siRNA targetting *JDP-2* exhibited reporter silencing of 60%, while the single-nucleotide mutant siRNA silenced reporting by 30%. The authors comment that the limited RNAi effects observed by themselves, as well as Elbashir *et al.* "were not completely in accord with the findings of more recent studies, possibly because of the different conditions used" (page 305), thereby acknowledging the uncertainty prevailing in contemporary research.

Finally, Applicants reiterate that Boutla *et al.* (*Current Biology,* 11: 1776-80, 2001) designed siRNAs in the same fashion as the Tuschl/Elbashir siRNAs, but obtained quite different results. Both the Tuschl/Elbashir experiment and the Boutla *et al.* experiment were designed to investigate how sequence changes within the oligonucleotide duplexes affected silencing/target recognition. Boutla *et al.* states "it was our intention to introduce a single nucleotide exchange that would interfere as much as possible with substrate binding" (see page 1779, column 1, paragraph 3, lines 2-4, emphasis added). Accordingly, Boutla *et al.* introduced a single, **centrally positioned mismatch** relative to the mRNA target sequence in each of the three mutant siRNA sequences (see Table 1(j), 1(k) and 1(l)). In fact, Boutla *et al.* introduced mismatches at the same position (P10) as the siRNAs in the Tuschl/Elbashir experiment. Nevertheless, despite the fact that they were designed in the same fashion as the Tuschl/Elbashir siRNAs, the siRNAs of Boutla retained significant efficacy against a mismatched target, leading Boutla *et al.* to conclude that "a perfect match to the target RNA is not necessary to initiate the RNAi response" (page 1779).

Collectively, the preceding references (Holen, Jacque *et al.*, Yu *et al.*, Hamada and Boutla *et al.*) suggest that the effect of single-nucleotide mismatches (including centrally-placed mismatches) was highly unpredictable at the time of the invention. Accordingly, one of skill in the art would have no reasonable expectation that a siRNA could discriminate amongst a mutant SOD1 sequence to which it is perfectly matched and a wild-type SOD1 sequence with which it forms a nucleotide mismatch, such that the mutant sequence is selectively inhibited. As such, it

would not have been obvious to one of skill in the art make the claimed invention with any reasonable expectation of success.

(b) The Cited Art does Not Teach or Suggest Single-Nucleotide Allelic Discrimination Amongst SOD1 Alleles at the Level Presently Claimed

It is also important to note that the Tuschl/Elbashir experiment does not teach or provide any evidence that a siRNA is capable of selectively silencing amongst target sequences which differ by a single nucleotide. The Tuschl/Elbashir experiment is a standard gene-specific silencing experiment, wherein sequence changes are introduced into the paired segments of siRNA duplexes to examine the effects of these sequence changes on the efficiency of silencing a single target gene sequence (a firefly luciferase reporter sequence). Both Tuschl *et al.* and Elbashir *et al.* report that "transversion of the AU base pair located opposite the predicted target RNA cleavage site or 1 nt further away from the predicted site prevented target RNA cleavage". However, aside from the single firefly luciferase target sequence, there is no indication that this siRNA retains the ablity to cleave other RNAs, including target RNAs to which it is perfectly matched. In fact, the Tuschl/Elbshir experiment provides no evidence that the introduction of a target mismatch in the center of the siRNA results in anything other than a "dead" or inactive siRNA. Accordingly, there is no reasonable basis for one of skill in the art to conclude that siRNAs of Tuschl/Elbashir would retain the ability to inhibit a mutant target allele while preserving the expression of a wild-type allele.

In the instant application Applicants have demonstrated for the first time that the RNAi agents of the invention are capable of discriminating amongst wild-type and mutant SOD1 alleles at the single nucleotide level. In fact, Applicants' working examples demonstrate that a significant level of discrimination is achievable both *in vitro* and *in vivo*. For example, Figure 1B indicates that mutant P10 siRNA was capable of cleaving at least 50% of mutant G85R SOD1 in a *Drosophila* embryo lysate assay while wild-type SOD1 mRNA levels in the same assay were virtually unaffected. This discrimination was even more pronounced in mammalian (HeLa) cells, where expression of mutant SOD1-GFP fusion protein was not detected by Western blot analysis following administration of P10 siRNA targeted to the G85R point mutation (see Figure 5, Example VI). Here again, P10 siRNA had no effect on the expression of

endogenous wild-type SOD1 levels in the same cell. Finally, allelic discrimination by shRNA containing the P10 siRNA sequence was observed in mice co-transfected with both a myc-tagged wild-type human SOD1 protein and a mutant SOD1-GFP fusion protein containing the G93A point mutation (see Figure 6, Example VII). Here the expression ratio in liver of SOD1 G93A-GFP to wild-type SOD1 was less than 0.25. To highlight the significant allelic discrimination obtained by Applicants, and to expedite prosecution of the application, claims 1 and 9 have been amended to require the limitation that *expression of the mutant target allele is selectively inhibited by at least 50% and expression of the wild-type allele is not inhibited.* As discussed in the Examiner Interview of January 20, 2010, neither Tuschl *et al.* nor Elbashir *et al.* teach or suggest that a modified siRNA retains a significant level of silencing (e.g., 50% or more) against a mutant target allele, without inhibiting expression of the wild-type allele.

In addition, Applicants submit that there appears to be nothing in the cited art which demonstrates successful or otherwise significant allele-specific SOD1 silencing using antisense technology. For example, Brown *et al.* (WO 94/19493) merely state in a prophetic example that a DNA oligonucleotide capable of binding with mutant SOD-1 mRNA may be designed. Similarly, the Klug *et al.* abstract (European Journal of Physiology, Vol. 441 (6 suppl 2): S27-S35, 1996) provides no evidence that significant SOD1 allelic discrimination was achievable with antisense technology. Although Klug *et al.* state that "[t]he aODN that was most G93A-specific *in vitro*...prevented expression of the mutant RNA four times more effectively than expression of WT RNA", they provide no data to backup this assertion. Thus, one of ordinary skill in the art has no way of determining whether the allelic discrimination, if any, was meaningful. For example, the description of the experiment conducted by Klug *et al.* could merely mean that expression of the mutant RNA was inhibited by 4% and expression of the WT RNA was inhibited by 1%. Even if one were to assume that the Klug *et al.* were able to achieve a significant level of allelic discrimination, the Klug *et al.* abstract implies that the SOD1 antisense oligonucleotide retained an undesirable capacity to silence the wild-type SOD1 allele. This contrasts sharply with the siRNA molecules of the invention which significantly inhibit expression of mutant SOD1 alleles but do not inhibit expression of the counterpart wild-type allele.

Not only do the Brown *et al.* and Klug *et al.* references fail to provide any evidence of significant allele-specific inhibition, but neither reference provides an enabling disclosure that would enable one of ordinary skill in the art to make or design an oligonucleotide capable of discriminating between mutant and wild-type SOD-1 alleles. As noted above, the Brown *et al.* reference is prophetic. Brown *et al.* provides no teaching of how to create a DNA oligonucleotide capable of single nucleotide discrimination. Although the antisense molecules of Klug *et al.* are purported to specifically silence mutant SOD1, Klug *et al.* is merely an abstract and lacks any of the details necessary for the skilled artisan to make and use the referenced antisense molecules. Accordingly, Applicants submit that the deficiencies in the teachings of Tuschl *et al.* and Elbashir *et al.* are not remedied by the antisense teachings of Brown *et al.*, Klug *et al.,* Siddique *et al.* and Kunst *et al.*[2]

Thus, in view of the above, Applicants respectfully request that the Examiner reconsider and withdraw the §103 rejection.

[2] Siddique *et al.* and Kunst *et al.* are relied on by the Examiner for teaching specific disease mutations in SOD1 and are otherwise irrelevant to the claimed invention.

CONCLUSION

Applicants believe the pending application is in condition for allowance. If a telephone conversation with Applicants' attorney would help expedite the prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 227-7400.

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Respectfully submitted,

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